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VINBLASTINE: INFLUENCE ON NERVE CONDUCTION
AND SYNAPTIC TRANSMISSION

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VINBLASTINE: Influence on Nerve Conduction and Synaptic Transmission

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Vinblastine injected into the eyes of pigeons had a detrimental effect on the amplitude of the postsynaptic evoked potential in the optic tectum; impulse conduction of the optic nerve was not influenced. The depression of the postsynaptic evoked potential was dependent on the dosage. Only 20 and 100 μg vinblastine had an influence; no influence was observed with 10 μg vinblastine. The reversible depression was seen from the third day after injection to between the 21st and the 28th day after injection, and in that period it remained constant. The latency of the response was not influenced by vinblastine.

INTRODUCTION

Colchicine inhibits axoplasmic transport and induces organelle alterations in axons and nerve terminals (Boesch et al., 1972; Cuénod et al., 1972; Davison, 1971; Edström and Mattsson, 1972; Hinkley and Samson, 1974; Hökfelt and Dahlström, 1971; Kreutzberg, 1969). The mechanism of this blockade may be explained by the hypothesis that colchicine interferes with the structural organization of the microtubules, which seem to be involved in the fast axoplasmic transport (Boesch et al., 1972; Edström and Mattsson, 1972; James et al., 1970; Karlsson and Sjöstrand, 1969; Kreutzberg, 1969; Schubert, 1972). Furthermore, the postsynaptic responses evoked by electrical stimulation of the pre-synaptic axons are reduced (Perisic and Cuénod, 1972). This deficit in synaptic transmission may be a consequence of the blockade of axonal flow.

Another drug which may be used to block axonal transport is vinblastine. As it is described in different papers (Bensch and Malawista, 1969; Bensch et al., 1969; Bunt, 1973a; Hinkley and Samson, 1972; Hinkley and Samson, 1974; Marantz et al., 1969; Weisenberg and Timasheff, 1970; Wilson et al., 1970; Wisniewski et al., 1968), vinblastine has an effect similar to colchicine on microtubular structure and axonal transport. Vinblastine also destroys already existing microtubules (Behnke, 1970; Bensch and Malawista, 1969; Bensch et al., 1969; Edström and Mattsson, 1972) and may inhibit protein synthesis in the perikaryon when applying high doses (Edström and Mattsson, 1972). These anatomical and chemical findings, showing a similarity with known data about colchicine, provide the background for the present study of the electrophysiological effects of vinblastine. The visual system of the pigeon was chosen as a model to test the influence of vinblastine on the nerve cell. The effect obtained at different time intervals after injection of vinblastine into the eye

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was examined by stimulation of the optic nerve, and recordings were made in the optic tract and optic tectum. In the optic tract and on the surface of the optic tectum impulse conduction could be examined. In the depth of the optic tectum, stratum griseum et fibrosum superficiale, stratum griseum centrale, and stratum album centrale (Cowan, 1970) recordings were made to test the influence of vinblastine on the postsynaptic evoked potentials. The normal responses in the optic tectum have been studied by, among others, Holden (1968) and Robert and Cuénod (1969). The advantage of this *in vivo* system is that one side can be used to test the direct influence of vinblastine and the other as a control to exclude systemic effects of vinblastine.

METHODS

The experiments were performed on 31 adult pigeons (*Columba livia*) of either sex.

Preparation

Vinblastine (Velbe) — in doses of 10 μg in 20 μl of Ringer solution, 20 μg in 20 μl , or 100 μg in 50 μl — was injected into the vitreous humor of one eye of each pigeon under Pentrane (methoxyfluorane) anesthesia. After various intervals the animals were anesthetized for the electrophysiological studies by intramuscular application of Equithesine (chloralhydrate and pentobarbital solution) 0.3 ml/100 mg of body weight. The trachea was cannulated and the animals paralyzed by Flaxedil (triiodoethylate of gallamine) and artificially ventilated. The EKG was continuously controlled. The animals were fixed in a head holder according to Karten and Hodos (1967). The posterior part of the telencephalon and the optic lobes were exposed bilaterally and the dura opened. For electrical stimulation the optic nerve papilla was exposed by removing the eyelids, the nictitating membrane, and the cornea and by aspirating the lens and the crystalline body.

Stimulation

For stimulation bipolar stainless steel electrodes (distance of the tips, 0.3 mm) were placed on the optic nerve papilla. Single square-wave pulses of 1 to 2 ma, 0.1 msec duration, and frequency of 0.3 or 100 per second were delivered by a Grass S-8 stimulator.

Recording

Evoked potentials were recorded with tungsten wire electrodes with tip diameters of 5–10 μm and 5–25 pf of capacitance. The electrodes were placed in the optic tecta (on the surface and at depths of 500, 1,000, and 1,500 μm) and then in the optic tracts. Signals were amplified 1,000 times with a Tektronix 122 preamplifier and displayed on a Tektronix storage oscilloscope (R5103N oscilloscope, P11 single-beam storage, 5A14N four-channel amplifier, 5A21N differential amplifier, 5B10N time base/amplifier).

Histological control

At the end of the experiment the animal was first perfused with 200 ml of Ringer solution and then with formalin solution composed of 4% phosphate-buffered formaldehyde (pH 7.2) and 0.32 M sucrose. The brains were frozen and frontally sectioned at 50 μm . Sections were stained following the Nissl technique.

RESULTS

The influence of vinblastine on the optical system was examined at three different sites: in the optic tract, on the surface of the optic tectum, and in the deeper structures of the optic tectum.

Vinblastine effect on impulse conduction

At 14 days after vinblastine injection unilaterally, the responses in the optic tract (Fig. 1B) did not show any remarkable differences between the control side and the vinblastine-injected one. Small differences in amplitude were explained by differences in recording sites. On the surface of the tectum (Fig. 1A) there were negative waves with normal amplitude and latency with all doses.

Vinblastine effect on postsynaptic responses

At 3 days after injection of vinblastine, contralateral to the injected eye the postsynaptic evoked potentials in the depth of the tectum were reduced in amplitude (Fig. 2), but there was no increase in latency. The decreased amplitude remained constant through all layers. There were small differences between animals with the same vinblastine doses. At a depth of 500 μm 3 days after injection the average reduction caused by 20 μg vinblastine was 64–70%. The latency of the diminished positive wave was never longer than that of the control side. On the experimental side, the late negative responses were reduced and sometimes even absent.

Figure 2 shows the effect of different doses of vinblastine. A depression of the postsynaptic evoked potentials was observed with doses of 20–100 μg vinblastine. The animals with 10 μg vinblastine did not show any influence of vinblastine on the amplitude or the latency of the responses in the tract or in the tectum.

The effect of 20 μg vinblastine on the postsynaptic evoked potentials is shown in Fig 3: there was a reversible depression and normal amplitudes were seen 21 days after injection. The depression remained from the 3rd to the 14th day after injection. Pigeons injected with 100 μg vinblastine (Fig. 3) showed a reversible depression of the amplitude of the postsynaptic evoked potentials which lasted from the 3rd to the 21st day after injection. Between the 14th and the 28th day after injection the depression of the amplitude diminished gradually. Normal evoked potentials were recorded again at the 28th day. Figure 3 shows that the depression of the amplitude was equal for 20 and 100 μg vinblastine in the period between the 3rd and the 14th day after injection. However, the recovery period lasted 14 and 28 days, respectively.

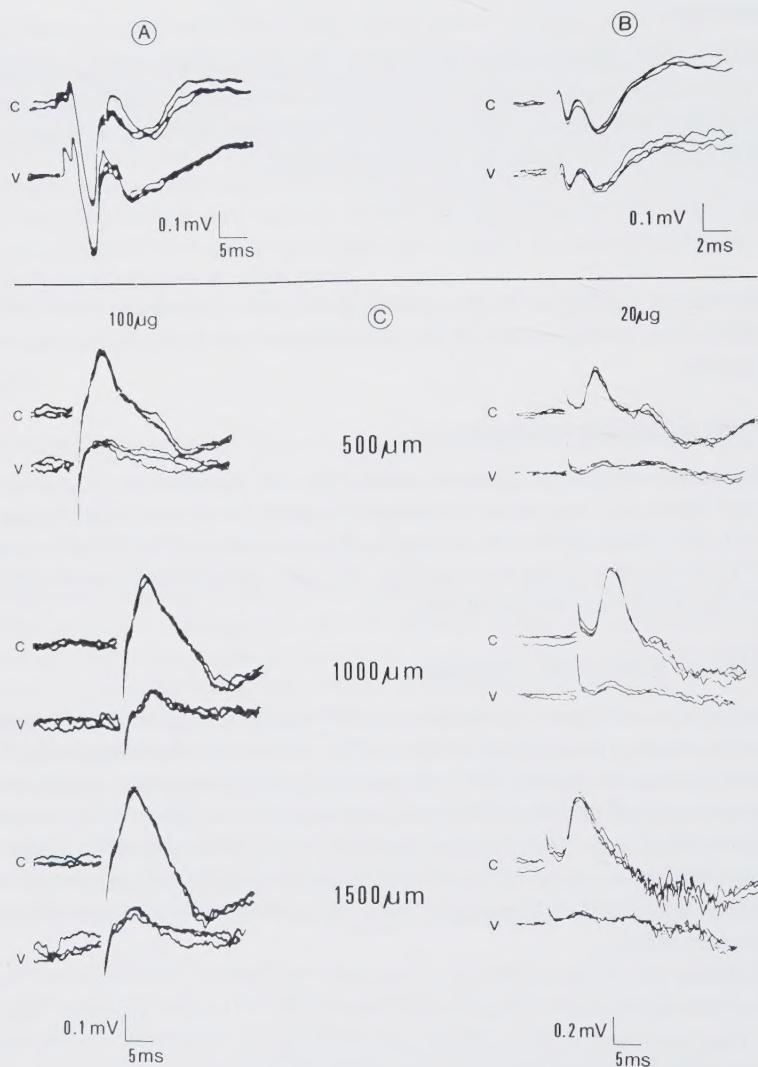


Fig. 1. Responses to electrical stimulation of the optic nerve: (A) on the surface of the optic tectum 3 days after injection of 100 μ g vinblastine into the right eye; (B) in the optic tract 14 days after injection of 20 μ g vinblastine into the right eye; (C) in the depth of the optic tectum (at 500, 1,000, and 1,500 μ m from the surface) 14 days after injection of 20 μ g or 100 μ g vinblastine into the right eye.

DISCUSSION

Vinblastine induces a reversible depression of postsynaptic evoked potentials within the optic tectum without apparently affecting nerve conduction in the optic tract (Fig. 1B). As the responses on the surface of the optic tectum are unaffected by vinblastine, one can argue that the small differences in the optic tract are caused by differences in the re-

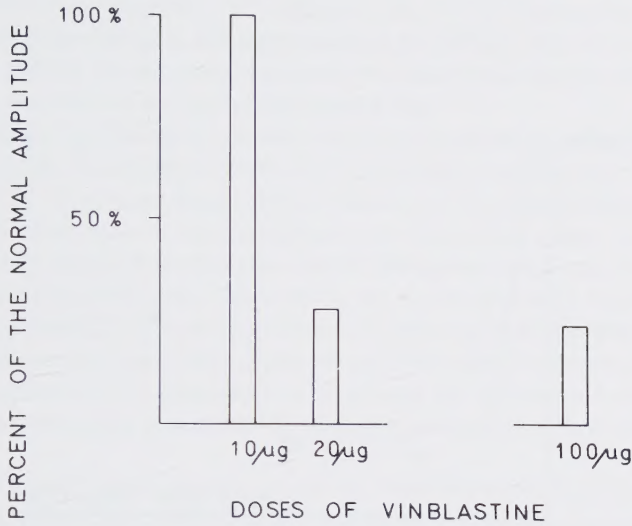


Fig. 2. Depression of the response in the optic tectum: vinblastine was injected into the right eye. Three days after injection the optic nerve was stimulated and recording was made at a depth of 1,000 μ m in the optic tectum.

cording sites. The argument that the responses in the optic tract are due to the antidromic firing of isthmooptic fibers can be disregarded, as those fibers constitute only 1% of all fibers in the optic tract (Cowan et al., 1961). Using another preparation, Dahlström et al., (1973) also observed an effect of vinblastine on synaptic transmission but not on impulse conduction. It seems, therefore, that vinblastine has no direct effect on nerve excitability between the papilla and the tectum. Conceivably, the membrane of the ganglion cell body and the initial portion of the optic nerve fiber could be affected. Such an effect at the site of vinblastine application could be suggested by the fact that vinblastine induces a functional lesion of the erythrocyte membrane (Seeman et al., 1973). As the stimulation in our experiments was performed in the papilla, this presumed effect should not interfere with our observations.

The reversible depression of the amplitude of postsynaptic evoked potentials can be explained in two ways: either by the effect of vinblastine on axonal transport or by a possible action of the drug directly on the terminal. It is known from several biochemical studies that vinblastine interferes with axonal flow (e.g., Edström and Mattsson, 1972). One interpretation is that by stopping the axonal flow of protein supplying nerve terminals, vinblastine impairs synaptic function, which returns only when vinblastine has disappeared from the system. It is known that vinblastine induces a disappearance of microtubules (Davison, 1971; Hinkley and Samson, 1972; Hökfelt and Dahlström, 1971; Tyson and Bulger, 1972; Wisniewski et al., 1968) and an increase in 100 Å diameter neurofilaments (Bunt, 1973a,d). Bunt mentions that rapid axonal flow is inhibited reversibly by 10 μ g vinblastine for 14 days. Furthermore this dose produced a noticeable decrease in numbers of microtubules in the ganglion cell perikarya and axons by one hr (Bunt, 1973c). Our experi-

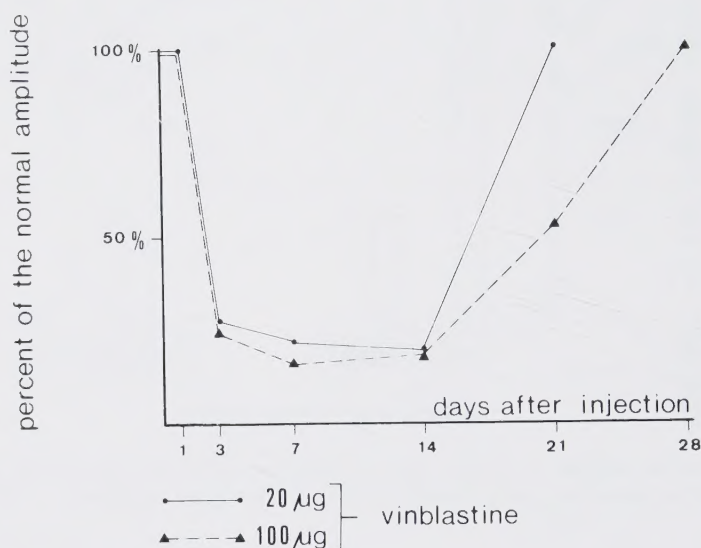


Fig. 3. The time course of the effect produced by the intraocular injection of 20 μ g or 100 μ g of vinblastine upon the evoked potential recorded at 1,000 μ m in the contralateral optic tectum of the injected eye.

ments suggest that 10 μ g vinblastine has no effect on synaptic transmission. The explanation of this apparent discrepancy could be that the arrest of axonal flow may not be complete, as there are still intact microtubules (Bunt, 1973d), and that therefore the remaining flow is sufficient to maintain synaptic function. Our results suggest, furthermore, that the time course for the inhibition of axonal flow with this dose of vinblastine is too short to influence synaptic transmission — that is, there is enough material in the nerve endings to maintain normal synaptic function until axonal flow is recovered.

Bunt (1973a) observed in the rabbit that the fast axonal flow failed to recover even at eight weeks after a dose of 25 μ g vinblastine or more, while in the pigeon reversible inhibition of axonal flow occurred after a dose of 20 μ g vinblastine (Cuénod et al., unpublished observations). In addition the physiological experiments showed a time course for reversible depression of postsynaptic evoked potentials (Fig. 3) even in pigeons treated with 20 or 100 μ g of vinblastine. These differences in results may be related to species differences.

Alternatively it is a possibility that vinblastine is transported to the synaptic region and induces alterations in systems other than the neurotubules. The depression of the postsynaptic response might also be due to an influence of vinblastine on the release of synaptic vesicles or an unspecific inactivation of proteins (Jokusch and Blessing, 1971;

Nicklas et al., 1973; Seeman et al., 1973; Wilson et al., 1970). However an inhibition of protein synthesis in the ganglion cell-soma seems to be unlikely after 10 to 100 μg vinblastine (Bunt, 1973c). On the present evidence we cannot say whether the electrophysiological effect is due only to the interrupted axonal flow.

Many authors describe paracrystalline inclusions in different cells after treatment with very high doses of vinblastine (Bunt, 1973a,d; Hinkley and Samson, 1972; Hinkley and Samson, 1974; Tyson and Bulger, 1972). Hinkley, 1974, showed in his *in vitro* experiments vinblastine-induced paracrystalline arrays in crayfish axons. As described by Bunt (1973a) they appear 4 hr after injection of 500 μg vinblastine into the eye of the rabbit and persist for several days. The doses in our experiment were smaller, and it is therefore perhaps unlikely that paracrystalline inclusions exist in the optic nerve endings. Also these inclusions are reported to appear much earlier than the depression of the postsynaptic response and to disappear at a time when the depression is still apparent. It seems unlikely that these paracrystalline inclusions directly interfere with synaptic transmission.

A different time course appears to exist for the depression of postsynaptic evoked potentials by colchicine and vinblastine. For colchicine it has been observed that there is a depression of postsynaptic evoked potentials one day after injection of 100 μg colchicine. One day after injection of 20 or 100 μg vinblastine there is no detectable effect. With vinblastine we have a complete restitution of the affected amplitude within 21 to 28 days (Fig. 3), whereas the effect of colchicine lasts 56 days. Other physiological differences between vinblastine and colchicine have been described by Katz, 1972; Turkanis, 1973; and Albuquerque et al., 1972). Furthermore, other investigations have shown (Edström and Mattsson, 1972; Hinkley and Samson, 1972; Nicklas et al., 1973; Owells et al., 1972; Shelanski, 1971; Weisenberg and Timasheff, 1970; Wilson, 1970; Wilson et al., 1970) that colchicine and vinblastine have different chemical properties. What they have in common is that they can bind to tubulin, but they do so at different sites (Shelanski, 1971; Wilson, 1970). It is debatable whether this differential binding reflects the different time course of events reported after vinblastine and colchicine injection into the avian eye. However, the fact that the intraocular injection of both colchicine and vinblastine affects the tectal postsynaptic response supports the hypothesis that this effect is mediated by the influence of the drugs on axoplasmic transport rather than on another system.

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